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A Ratiometric Fluorescent Chemodosimeter with Selective Recognition for Sulfite in Aqueous Solution

Yimin Sun, Cheng Zhong, Rui Gong, Honglei Mu, and Enqin Fu*

Department of Chemistry, Hubei Key Laboratory on Organic and Polymeric Optoelectronic Materials, Wuhan University, Wuhan 430072, P. R. China

fueq@whu.edu.cn

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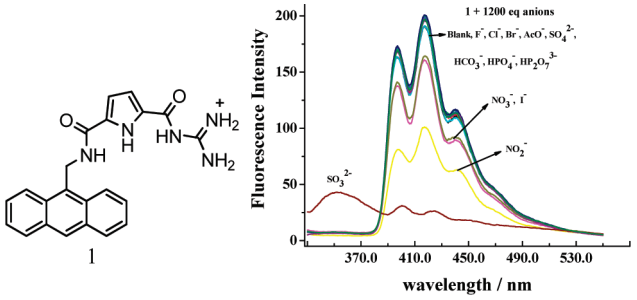

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A fluorescent chemodosimeter containing a guanidiniocarbonylpyrrole and a 9-(aminomethyl)anthracene moiety has been synthesized. The sensor exhibits ratiometric fluorescence changes for SO_3^{2-} over other anions in 90% water/DMSO. The interesting ratiometric fluorescent changes for SO_3^{2-} are attributed to the fluorescence resonance energy transfer (FRET) and the SO_3^{2-} complex induced photochemical reaction.

The development of fluorescent chemosensors for anions has received considerable attention due to the simplicity and sensitivity of fluorescence.[1] However, the conventional fluorescence methodology, which monitors the fluorescence intensity at a single wavelength, is easily interfered by sensor concentration, photobleaching, optical path length, and illumination intensity.[2] To eliminate those effects, a ratiometric fluorescent measurement is desirable. This technique uses the ratio of the fluorescent intensities at two different wavelengths, allowing precise and quantitative analysis and imaging even in complicated systems.[3] A variety of signaling mechanisms such as intramolecular charge transfer (ICT),[4] metal−ligand charge transfer (MLCT),[5] excimer/exciplex formation,[6] and fluorescence resonance energy transfer (FRET)[7] can be employed for the design of ratiometric measurement. Among them, FRET is a mechanism that is commonly used for ratiometric signaling for analytes due to its potential practical benefits in cell physiology, optical therapy, as well as selective and sensitive sensing toward target molecular or ionic species.[8] Many efforts have been made to design FRET-based ratiometric fluorescence for cations in recent years,[9] but the ratiometric fluorescent chemosensor for anion is still rare,[10] especially for those anions with deleterious effects on the environment[11] in aqueous solution.

Among the various anionic analytes, sulfite is of considerable interest due to the significant amount of sulfur dioxide released in industrial process and its deleterious effects on the environment.[12] The traditional method of sulfite sensing is ion-selective electrodes.[13] In the past, some guanidinium-based ion-selective electrodes for sulfite have been reported,[14] and the response behavior indicates the existence of a selective interaction between the guanidinium moiety and the sulfite anion.[15]

In this paper, we present a new FRET-based ratiometric fluorescent chemodosimeter **1** for sulfite, in which guanidiniocarbonyl pyrrole moiety[16] is covalently attached to

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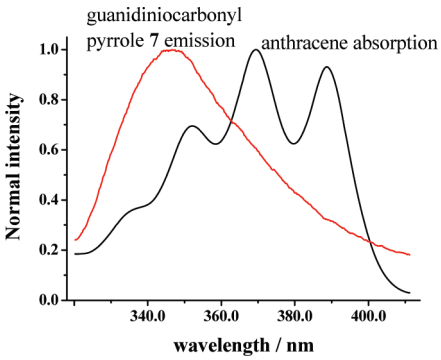
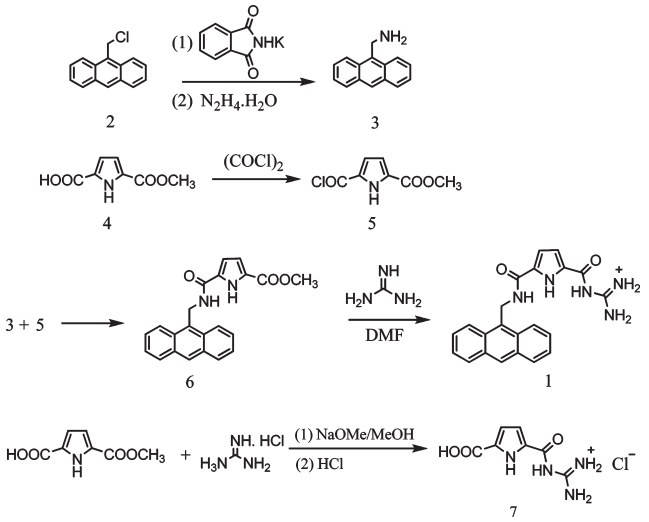


FIGURE 1. Spectral overlap of the guanidiniocarbonyl pyrrole emission with the anthracene absorption.

SCHEME 1. Synthesis of Compound 1 and Reference Material 7



9-(aminomethyl)anthracene. The spectral overlap between guanidiniocarbonyl pyrrole emission and anthracene absorption makes FRET possible (Figure 1). In the presence of sulfite, receptor **1** interacts with sulfite through a combination of ion pairing and multiple hydrogen bonds, and then upon irradiation, the anthracene moiety undergoes an intermolecular [4π + 4π] photodimerization. This minimizes the spectral overlap between the donor emission and the acceptor absorption bands, resulting in the cancellation of the FRET effect between the two fluorophores. The combined interaction makes **1** show a ratiometric fluorescence change for SO_3^{2-} over other anions including F^-, Cl^-, Br^-, I^-, AcO^-, SO_4^{2-}, HCO_3^-, HPO_4^{2-}, $P_2O_7^{4-}$, NO_3^-, and NO_2^-. Under the conditions of fluorescent titration, the special spectroscopic properties are shown to be related to sulfite concentration, so receptor **1** could be used for the quantitative analysis of sulfite. To the best of our knowledge, receptor **1** is the first ratiometric fluorescence chemodosimeter induced by photochemical reaction. Meanwhile, it should be noted that sulfite-induced anthracene photodimerization has not been reported so far.

Receptor **1** was synthesized according to Scheme 1. Pyrroledicarboxylic acid monomethyl ester **4** was converted into the acyl chloride **5** by reaction with oxalyl chloride in CH_2Cl_2 in the presence of a catalytic amount of DMF. Without further purification, the crude acyl chloride then reacted with 9-(aminomethyl)anthracene **3** to give **6** in 70% yield. The guanidinylation of ester **6** was achieved by refluxing the ester and an excess of guanidine in dry DMF under nitrogen. Upon acidification with hydrochloric acid, the hydrochloric salt of receptor **1** precipitated in 40% yield. After reaction with picric acid, the picrate salt of receptor **1** was obtained as yellow powder.

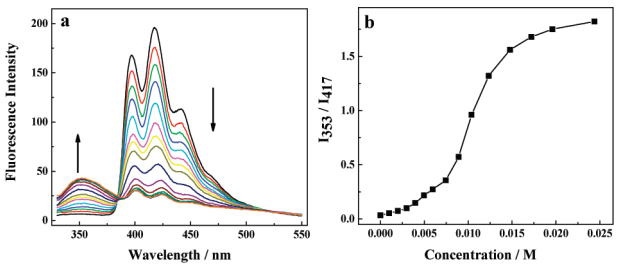


FIGURE 2. (a) Fluorescence emission changes of **1** (picrate salt, 2.0×10^{-5} M) upon addition of sodium sulfite (0−1500 equiv) in 90% water/DMSO solution (pH 7.2, 10 mM Tris−HCl buffer). (b) Ratiometric calibration curve of I_{417}/I_{353} as a function of sulfite concentration.

The photophysical properties of receptor **1** were first studied in 90% water/DMSO solution (10 mM Tris−HCl buffer, pH = 7.2). The absorption spectra of **1** displayed three characteristic bands of anthracene appearing at 348, 366, and 386 nm, and extinction coefficients were determined to be 5250, 7650, and 6900 M^{-1} cm^{-1}, respectively. When excited at 290 nm (the excitation wavelength for guanidiniocarbonyl pyrrole), receptor **1** would emit fluorescence derived from anthracene at 417 nm, which suggested that intramolecular FRET occur effectively between the two adjacent fluorophores. Using anthracene as reference (Φ_F = 0.27 in EtOH, λ_{ex} = 365 nm), the quantum yield of receptor **1** was determined as 0.16.

The recognition properties of **1** were studied in 90% water/DMSO (10 mM Tris−HCl buffer, pH = 7.2) with various anions as substrates by fluorescence spectroscopy. After the addition of sodium sulfite (0−29 mM) to receptor **1** (picrate salt, 2.0×10^{-5} M), the fluorescence intensity derived from anthracene greatly decreased (Φ_F = 0.012), while the fluorescence intensity at 353 nm derived from guanidiniocarbonylpyrrole increased with an isoemissive point at 382 nm, which made receptor **1** serve as a ratiometric fluorescent chemodosimeter for SO_3^{2-} (Figure 2). The complex formation constant K_a for the **1**−sulfite complex was calculated to be 104 M^{-1}, and the job analysis indicated that receptor **1** formed a 1:1 complex with sulfite (Supporting Information, Figures S1 and S2). Addition of nitrite, nitrate, iodide, and bromide also caused fluorescence decrease of the anthracene moiety, but no fluorescence enhancement at 353 nm was observed (Supporting Information, Figures S3 and S4). In the presence of nitrite (160 mM) and nitrate (330 mM), the quantum yields of receptor **1** came to be 0.0036 and 0.017 respectively. The binding constants of receptor **1** to NO_2^- and NO_3^- were determined to be 35 and 7 M^{-1}.Other anions such as F^-, Cl^-, AcO^-, SO_4^{2-}, HCO_3^-, HPO_4^{2-}, and $P_2O_7^{4-}$ did not affect emission intensity significantly.

To get a further insight of the ratiometric fluorescent changes of **1**, UV−vis titration was performed under the same conditions as the fluorescence titrations (Figure 3). The

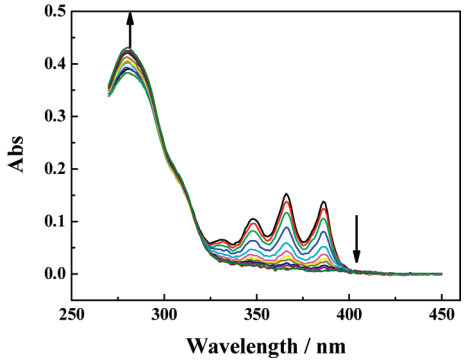


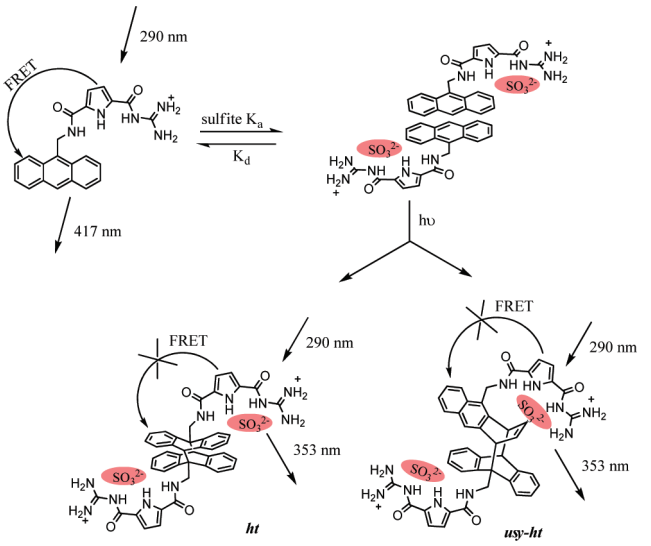
FIGURE 3. Absorption spectrum of receptor **1** (hydrochloride salt, 2.0×10^{-5} M) upon the addition of SO_3^{2-} in 90% water/DMSO solution (pH 7.2, 10 mM Tris−HCl buffer).

hydrochloride salt of **1** was employed to eliminate the absorption overlap of picrate and anthracene. We found that the decrease of anthracene's absorption spectra (350−420 nm) was accompanied by that of the fluorescence spectrum, while other anions did not affect the absorption spectrum of receptor **1** (Supporting Information, Figure S5). Subunit **3** showed a typical PET behavior upon sulfite recognition, and the emission and absorption spectra of subunit **7** did not change upon the addition of sulfite. Thus, we propose that the absorption and fluorescence change of anthracene in receptor **1** is ascribed to its structure change upon irradiation. Gunnlaugsson[17] and Ito[18] have described that the symmetric and unsymmetric [$4\pi + 4\pi$] photocycloaddition dimerization reactions are carried out between two anthracene derivative molecules upon irradiation. On the basis of the above facts, a possible explanation for the ratiometric fluorescence changes is that the binding of sulfite enhances the rate of the photodimerization reaction of receptor **1**. When the sulfite is added, receptor **1** forms a 1:1 complex with sulfite. The binding of sulfite reduces mobility of receptor **1**, and the $\pi-\pi$ interaction between the anthracene moieties makes this 1:1 complex become 2:2 complex. Upon irradiation, the 2:2 complex undergoes a well-established [$4\pi + 4\pi$] cycloaddition reaction of anthracene (Scheme 2).

The origin of the fluorescence quenching by nitrite ions is possibly ascribed to the reasons below. The absorption band of nitrite coincides with the emission band of guanidiniocarbonyl pyrrole, and this makes the FRET between guanidiniocarbonylpyrrole and anthracene less effective; as a result, the fluorescence intensity of anthracene decreased. In this case, the ground state of anthracene is not affected and the emission light of guanidiniocarbonyl pyrrole is absorbed either by nitrite ion or by anthracene, so the fluorescence of the guanidiniocarbonyl pyrrole group is not present. The fluorescence quenching of nitrate ion is almost the same as that of the nitrite ions. The fluorescence decrease of receptor **1** upon addition of iodide and bromide are ascribed to the "heavy atom effect". Compared with iodide, the quenching extent of bromide is smaller.

To confirm the photodimerization mechanism proposed above, photoreactions of receptor **1** with sulfite were

SCHEME 2. Proposed Photochemical Reaction Mechanism of Receptor 1 with SO_3^{2-}



performed with a high-pressure mercury lamp (150 W) for 10 h in D_2O/DMSO-d_6 (4:6, v/v). ^{1}H NMR, mass spectrometry, and liquid chromatography experiments had been carried out for the detection of the photoproducts. From the ^{1}H NMR spectrum we found that the anthracene proton signals [δ 8.54 (s, 1H, An-H_{10}), δ 8.32 (d, 2H, An-H_4, H_5), δ 8.08 (d, 2H, An-H_1, H_8)] disappeared gradually, while new peaks appeared at δ 8.01, 7.60−7.70, and 7.21−7.40 (Figure 4). The new multiple peaks at a range of δ 7.21−7.40 were assigned to the protons of the *o*-xylene system,[19] and the new peaks at δ 8.01 and 7.60−7.70 were possibly assigned to the proton of naphthalene (H_4, H_5, H_8) obtained in the unsymmetric photoreaction. The change of the ^{1}H NMR spectrum was consistent with the appearance of the two photoproducts, the reaction mixture formation of ***ht*** and ***usy-ht*** anthracene photodimers. The mixture was analyzed by LC using a C18 reversed-phase column, and the chromatogram exhibited two main product peaks at the longer retention time, which were assigned to ***ht*** and ***usy-ht*** anthracene photodimers, respectively (Supporting Information, Figure S6). We then diluted this NMR sample to 2.0×10^{-5} M, and the UV−vis and fluorescence spectrum were performed. The spectroscopy changes were the same as those of the UV−vis and fluorescence spectra during the fluorescence titration (Supporting Information, Figure S7), so we proposed that the photoreactions under irradiation with a mercury lamp and under fluorescence titration underwent the same mechanism. The photoproduct−SO_3^{2-} complexes were also detected by the ESI-MS spectrum in the negative-ion mode. The peaks of m/z at 466 were assigned as $[M + 2SO_3]^{2-}$ (Supporting Information, Figure S8). Meanwhile, the 1:1 complex between sulfite and the chemodosimeter could be identified by this peak.

The experimental results show that the fluorescence decrease of anthracene moiety should be ascribed to the SO_3^{2-} complex induced photodimerization. Upon the addition of SO_3^{2-}, the fluorescence of anthracene moiety decreases

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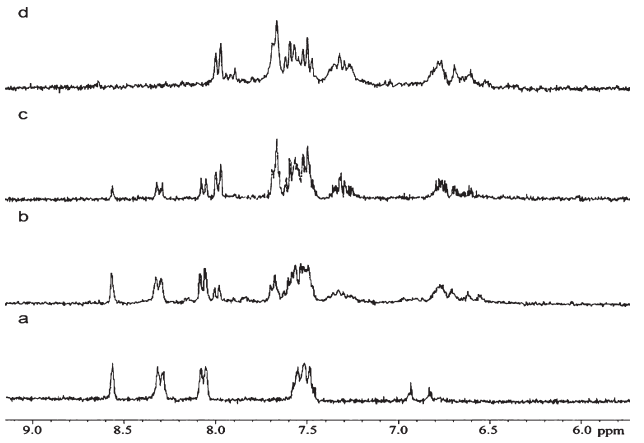

FIGURE 4. ^{1}H NMR of the photodimerization process of receptor **1** in the presence of sulfite under a mercury lamp: (a) 0 h; (b) 2 h; (c) 5 h; (d) 10 h.

gradually, and the guanidiniocarbonyl pyrrole emission at 353 nm will increase concomitantly; this can be referred to the FRET-off effect induced by the minimized spectral overlap between the donor emission and the acceptor absorption band. Hence, the fluorescence decrease resulted from the $[4\pi + 4\pi]$ photodimerization reaction of anthracene combined with the fluorescence enhancement of guanidiniocarbonyl pyrrole make receptor **1** serve as a ratiometric fluorescent chemodosimeter for sulfite.

The photochemical reaction is a common phenomenon for 9-substituted anthracene derivatives. Receptor **1** was found to be dimerized spontaneously in DMSO under room temperature, but the complete conversion to photodimerized product needed a time scale of almost half a year (Supporting Information, Figure S9). During the fluorescence measurement, the fluorescent spectrum of **1** without sulfite was affected only slightly by irradiation with the excitation light at 1 min intervals for 1 h (Supporting Information, Figure S10). In the presence of sulfite, the photocyclization of **1** was accelerated by sulfite complexation, and the fluorescence emission was greatly altered. Here we should notice that the rate of the photochemical reaction depended on two processes: the association process and the photodimerization process (as shown in Scheme 2). The association process depends on the concentration of sulfite, and the photodimerization of anthracene depends on the time, wavelength, and intensity of irradiation. During the process of the fluorescence titrations, the wavelength and the intensity of the excitation light are stationary and the titration was finished in a relatively shorter time (the measurement times are rigorously controlled within 10 min), so the fluorescence changes are mainly related to the sulfite concentration. Under this condition, in the absence of interfering ions (NO_2^-, NO_3^-, I^-), the ratio of pyrrole to anthracene emission can be used for the quantitative analysis of sulfite, and this chemodosimeter has a detection limit of 7.8×10^{-4} M in terms of sulfite. While in the presence of interfereferring ions, receptor **1** also exhibits good selectivity for sulfite and can be used for the qualitative analysis of sulfite (Figure 5).

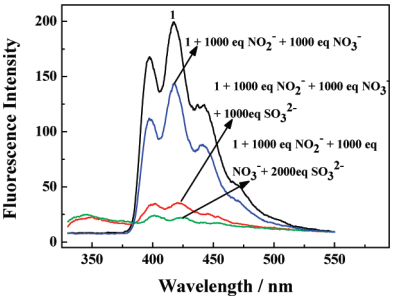

FIGURE 5. Fluorescence emission changes of receptor **1** (picrate salt, 2.0×10^{-5} M) with NO_2^-, NO_3^- (1000 equiv) upon addition of sulfite in 90% water/DMSO solution (pH 7.2, 10 mM Tris−HCl buffer).

In conclusion, we have synthesized a ratiometric fluorescent chemodosimeter for sulfite for the first time. The receptor exhibits a remarkable selectivity for sulfite in aqueous solution which could be used for the quantitative and qualitative analysis of sulfite. The FRET combined with the anion-complex sensitized photodimerization reaction is responsible for the recognition process.

Experimental Section

The Synthesis of 1. Ester **6** (358 mg, 1 mmol) and guanidine (5 equiv, prepared from guanidinium hydrochloride with sodium methoxide) were refluxed in 20 mL of dry DMF for 36 h under nitrogen. The orange solution was poured into water, and upon acidification with hydrochloric acid (2 M), the crude product precipitated. It was filtered off and washed thoroughly with methanol to provide hydrochloride salt of **1** (170 mg, 40%) as a brown solid. The picrate salt **1** was obtained by dissolving the hydrochloride salt in an aqueous picric acid solution and recrystallized from methanol as a yellow powder: mp 176−178 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.37 (s, 1H, pyrrole NH), 10.94 (s, 1H, guanidinium amide NH), 8.78 (s, 1H, carbamoyl NH), 8.64 (s, 1H, An-H_{10}), 8.56 (s, 2H, picrate), 8.41 (d, 2H, An-H_4, H_5), 8.13 (d, 6H, An-H_1, H_8, guanidinium NH_2), 7.58 (m, 4H, An-H_2, H_3, H_6, H_7), 6.99 (s, 1H, pyrrole CH), 6.92 (s, 1H, pyrrole CH), 5.48 (d, 2H, $-CH_2NH$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 35.9, 114.4, 115.6, 124.9, 125.4, 125.9, 126.0, 127.2, 128.3, 129.5, 129.6, 130.7, 131.7, 132.6, 142.2, 155.1, 159.3, 159.9; ESI-MS m/z = 386 ($M + H^+$). Anal. Calcd for $C_{28}H_{22}N_8O_9$: C, 54.73; H, 3.61; N, 18.23. Found: C, 54.64; H, 3.65; N, 18.14.

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Supporting Information Available: Synthesis and characterization of **6** and **1**, Figures S1−S15. This material is available free of charge via the Internet at http://pubs.acs.org.